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**Melanocytes derived from transgene-free human induced pluripotent stem cells.**

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**Public Summary:**

Defects in melanocytes have been implicated in the etiology of a variety of human skin diseases and disorders. An in vitro system that reliably and efficiently produces normal human melanocytes from embryonic stage cells would allow us to better dissect the physiological and pathological development of melanocytes. We have established a strategy to produce human melanocytes in vitro for use as a platform for pigment cell research and the development of cell-based therapies. We first derived transgene-free hiPSCs from two distinct types of skin cells, human primary melanocytes and human dermal fibroblasts, and obtained high-purity melanocytic derivatives from these hiPSCs using two newly-developed differentiation protocols. The differentiated derivatives possess molecular features of bona fide melanocytes and accurately mimic their ability to respond to  $\alpha$ -MSH. In addition, the cellular genome of these derivatives remained stable during reprogramming and differentiation. Similar to human melanocytes, the differentiated derivatives integrated well in semi-autologous skin reconstructs. Like the autologous dermal fibroblasts used for generating transgene-free hiPSCs, the differentiated derivatives stimulated limited proliferation of peripheral blood mononuclear cells that were isolated from the blood of the same individual in a mixed lymphocyte reaction assay. In this study, we have demonstrated that genetically stable melanocytes can be efficiently differentiated from transgene-free hiPSCs generated from two different types of cutaneous cells. Our approach can serve as an unlimited source of custom human melanocytes that can be used for novel approaches for modeling human skin disease and to provide material for transplantation.

**Scientific Abstract:**

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